PATENT
Attorney Docket No: AURO1130-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Negulescu *et al.*	Art Unit:	1647
Serial No.	:	09/468,002	Examiner:	R. Landsman
Filed	:	December 20, 1999		
Title	:	PROMISCUOUS G-PROTEIN COMPOSITIONS AND THEIR USE		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

The undersigned, Mel Simon, Ph.D., does hereby declare as follows:

1. I am a co-inventor of the invention claimed in the above-referenced patent application.

2. I am Chairman and Professor of Biology at the California Institute of Technology, Pasadena, California 91125 and member of the National Academy of Sciences since 1985. My *curriculum vita is* attached.

3. One of my central areas of research is G-protein coupled receptors and G-proteins. Accordingly I believe that I have at least ordinary skill in the art in this area of research.

4. I am familiar with the Office Action setting forth rejections of claims.

5. I am also familiar with Pausch *et al.* (U.S. Patent No. 5,691,188). ("Pausch *et al*") cited in the Office Action relating to G protein expression, Offermans *et al.* (J. Biol. Chem. 270 15175-15175, 1995) ("Offermans *et al.*") relating to transient over expression of G-proteins, Abe *et al.* (J. Biol. Chem. 268: 12033-12039) ("Abe *et al.*") relating to the expression of gustatory G-protein coupled olfactory receptors, Negulescu *et al.* (PNAS 91: 2873-2877, 1994) ("Negulescu *et al.*"), relating to the intracellular calcium dependence of gene expression, Hazlett *et al.*, (Biochemistry 32: 13575-13583, 1994) ("Hazlett *et al.*") relating to the solution dynamics of p21 ras proteins bound to fluorescent nucleotides, and Goddard et al. (ISLAR 1992

Proceedings, pages 392-399) relating to automated high throughput cell based drug screening systems.

6. I have personal knowledge of unpublished experiments in my laboratory based on Gα protein (15 and 16) expression and G-protein coupled receptors. Our initial experiments attempting to make permanent cell lines with Gα proteins with promiscuous coupling properties were impeded by the difficulty of over expressing the Gα proteins (15 and 16). In these experiments, we screened hundreds of clones. In such cells over expression created two impediments: 1) toxicity to the cells or 2) down regulation of G-protein coupled receptors. This impeded coupling of Gα proteins to G-protein coupled receptors or assay development.

7. I and my co-inventors have also demonstrated that efficient G-protein coupling to G-protein coupled receptors is hampered by insufficient expression of G-proteins. For example the attached figure entitled "Gα15 Expression levels (Western blot Analysis) correlate with Promiscuous Coupling", demonstrates that low levels of Gα15 expression, as shown with clone PN4-44, is not sufficient for promiscuous coupling.

8. I believe that the claimed invention is not obvious in light of the cited references because one-skilled in the art would not know whether stable expression of a G protein in a cell would be either 1) excessive and therefore cause toxicity or down regulation or 2) be insufficient for promiscuous coupling. Specifically Offermans *et al.* describes only the transient expression of Gα15, and provides no guidance as to the production of stably expressing cell lines, or the ability of Gα15 to enable promiscuous coupling of GPCRs when stably expressed within a cell.

9. I, and my co-inventors, created useful stable cell lines by functionally selecting them using a signal transduction detection system as described in the above-referenced patent application. Stable cells can be generated that tolerate the expression of a signal transduction coupling protein (e.g. a promiscuous G protein) and/or a G-protein coupled receptor. Such signal transduction detection systems include transcriptional-based assays and intracellular ion assays and can also be tested with either endogenously expressed or heterologously expressed GPCRs in cells expressing a promiscuous Gα protein. By employing fluorescent detection methods as taught in the above referenced patent application, a practitioner can also characterize a single cell. Accordingly, convenient cell-sorting methods, such as FACS, can be used to rapidly analyze and isolate cells that express promiscuous G-protein for functional coupling.

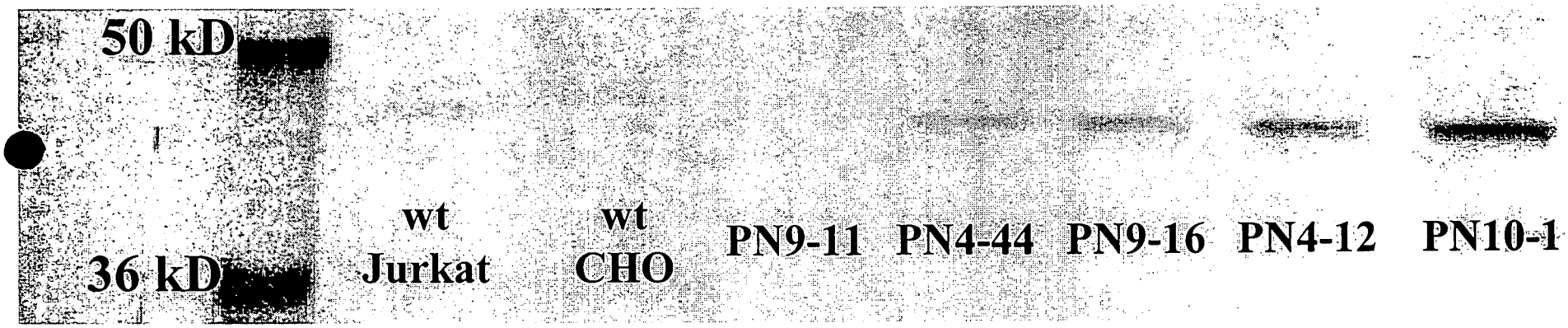
DECLARATION

I declare that all statements made here of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: July 29 2001

By:

Gα15 Expression Levels (Western Blot Analysis) Correlate with Promiscuous Coupling



nd	-	nd	-	-/+	+	+

Response to non Gq-type GPCRs:

ND = Not determined
\- = no promiscuous coupling
-/+ = coupling to some tested receptors
\+ = coupling to all receptors tested

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Curriculum Vitae

Melvin I. Simon

Date of Birth: February 8, 1937, New York

Social Security No.: 112-28-1210

Educational Background:

B.S., Chemistry (1959), The City College of New York, New York, New York

Ph.D., Biochemistry (1963), Brandeis University. Waltham, Massachusetts

Research/Professional Experience:

1995-present	Chairman and Professor, Division of Biology, California Institute of Technology, Pasadena, California 91125
1982-1995	Professor, Division of Biology, California Institute of Technology, Pasadena, California 91125
1976-1982	Professor, Department of Biology, University of California, San Diego, La Jolla, California 92093
1970-1976	Associate Professor, Department of Biology, University of California, San Diego, La Jolla, California 92093
1965-1970	Assistant Professor, Department of Biology, University of California, San Diego, La Jolla, California 92093
1964-1965	Postdoctoral Fellow, Princeton University, Princeton, New Jersey 08544

Advisory Boards:

1980-present	Chairman, Board of Directors, Agouron Institute, La Jolla, California
1984-1989	Member of the Board of Visitors, Massachusetts Institute of Technology
1984-1992	Board of Governors, Technion, Israel Institute of Technology, Haifa, Israel
1984-1992	National Scientific Advisory Board, Douglas French Foundation for Alzheimer's Disease
1985-1990	National Scientific Advisory Board, Hereditary Disease Foundation
1985-present	Member of the Board of Directors, Agouron Pharmaceuticals, Inc., La Jolla, California
1986-present	Co-editor of *Methods in Enzymology*
1986-1991	Advisory Board, Biological Sciences Division, Office of Naval Research
1988-1992	Advisory Panel, Microbial Genetics, National Institutes of Health
1988-1994	Medical Advisory Board, Howard Hughes Medical Institute
1988-1995	Advisory Board, Hutchinson Cancer Foundation
1989-1992	J. D. Searle Fellowship Review Committee

1989-present	Scientific Advisory Board, La Jolla Cancer Research Foundation
1989-present	Co-editor of *Methods - A Companion to Methods in Enzymology*
1990-present	Department of Energy's Office of Health and Environmental Research Advisory Committee (HERAC)
1990-present	Editorial Board, *Molecular Microbiology*, *Molecular Biology of the Cell*, and *Marine Molecular Biology*
1993-1994	Eli Lilly Award Selection Committee, American Society of Microbiology
1993-1994	Scientific Advisory Board, CADUS Pharmaceutical Corp., New York
1993-present	Department of Energy, Human Genome Project Coordinating Committee
1994-present	Scientific Advisory Board and Board of Directors, Industrial BioCatalysis, Inc.
1994-present	Adjunct Professor of Cell and Neurobiology, University of Southern California School of Medicine, Los Angeles, California
1995-present	Board of Governors, American Academy of Microbiology
1995-present	Board of External Scientific Advisors, Childrens Hospital Los Angeles Research Institute
1996-present	Science and Technology (S&T) Panel of the President's Council on the National Laboratories, UC/DOE

Awards:

John Simon Guggenheim Memorial Fellowship, 1978

Member, National Academy of Sciences, 1985

Member, American Academy of Arts and Science, 1986

Anne P. and Benjamin F. Biaggini Professor of Biological Sciences, 1987

Selman A. Waksman Award in Microbiology, National Academy of Sciences, 1991

Recent Endowed Lectures:

Carter-Wallace Lecturer, Princeton University, 1988

Robert A. Welch Foundation Lecturer, Houston, 1989

Frontiers in Cell Biology, Columbia University, 1989

Frontiers in Biology Lecturer, Texas A & M, 1990

Burroughs-Wellcome Lecturer, Kalamazoo College, 1990

Lucille P. Markey Lecturer, University of California, Berkeley, 1991

Tracy Sonneborn Memorial Lecturer, Indiana University, 1992

Storer Life Sciences Lecturer, University of California, Davis, 1992

Nathan Kaplan Memorial Lecture. Brandeis University, 1993

Steinberg/Wylie Lecture, University of Maryland, 1993

Publications:

1. Simon, M. I. and H. Van Vunakis. The photodynamic reaction of methylene blue with DNA. *J. Mol. Biol.* **4**, 488, 1962.

2. Kallen, R., M. I. Simon and J. Marmur. The occurrence of a new pyrimidine base replacing thymine in a bacteriophage DNA. *J. Mol. Biol.* 5, 248-250, 1962.

3. Heally, W., D. Stollar, M. I. Simon and L. Levine. Characterization of a phosphodiesterase from lamb brain. *Arch. Biochem. Biophys.* **103**, 461, 1963.

4. Simon, M. I. and H. Van Vunakis. The dye-sensitized photooxidation of purine and pyrimidine derivatives. *Arch. Biochem. Biophys.* **105**, 197-206, 1964.

5. Simon, M. I., L. Grossman and H. Van Vunakis. Photosensitized reaction of polyribonucleotides. I. Effects on their susceptibility to enzyme digestion and their ability to act as synthetic messengers. *J. Mol. Biol.* **12**, 50-59, 1965.

6. Simon, M. I. Photosensitized oxidation of purines and pyrimidines. In: *Methods in Enzymology*, Vol. XII, Part A, L. Grossman and K. Moldave, eds., Academic Press, Inc., New York, pp. 45-47, 1967.

7. Simon, M. I. Photosensitization. In: *Comprehensive Biochemistry*, Vol. 27, M. Florkin and E. H. Stotz, eds., Elsevier Publishing Co., Amsterdam, pp. 137-156, 1967.

8. Grant, G. P. and M. I. Simon. Use of radioactive antibodies for characterizing antigens and application to the study of flagella synthesis. *J. Bacteriol.* **95**, 81-86, 1968.

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11. Van Alstyne, D., G. F. Grant and M. I. Simon. Synthesis of bacterial flagella: Chromosomal synchrony and flagella synthesis. *J. Bacteriol.* **100**, 283-287, 1969.

12. Emerson, S. U., K. Tokuyasu and M. I. Simon. Bacterial flagella: Polarity of elongation. *Science* **169**, 190-192, 1970.

13. Dimmitt, K. and M. I. Simon. Antigenic nature of bacterial flagellar hook structures. *Infection and Immunity* **1**, 212-213, 1970.

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15. Dimmitt, K. and M. I. Simon. Purification and thermal stability of intact *Bacillus subtilis* flagella. *J. Bacteriol.* **105**, 369-375, 1971.

16. Colby, C., B. D. Stollar and M. I. Simon. Interferon induction: DNA-RNA hybrid or double stranded RNA? *Nature New Biol.* **229**, 172-174, 1971.

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19. Dimmitt, K. and M. I. Simon. Purification and partial characterization of *Bacillus subtilis* flagellar hooks. *J. Bacteriol.* **108**, 282-286, 1971.

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75. Boyd, A., A. Krikos, N. Mutoh and M. I. Simon. A family of homologous genes encoding sensory transducers in *E. coli*. In: *Mobility and Recognition in Cell Biology*. H. Sund and C. Veeger, eds., Walter de Gruyter, Berlin, pp. 551-562. 1983.

76. Silverman, M. and M. I. Simon. Phase variation and related systems. In: *Mobile Genetic Elements*, James A. Shapiro, ed., Academic Press, New York, pp. 535-555, 1983.

77. Szekely, E. and M. I. Simon. DNA sequence adjacent to flagellar genes and the evolution of flagellar phase variation. *J. Bacteriol.* **155**(1), 74-81, 1983.

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